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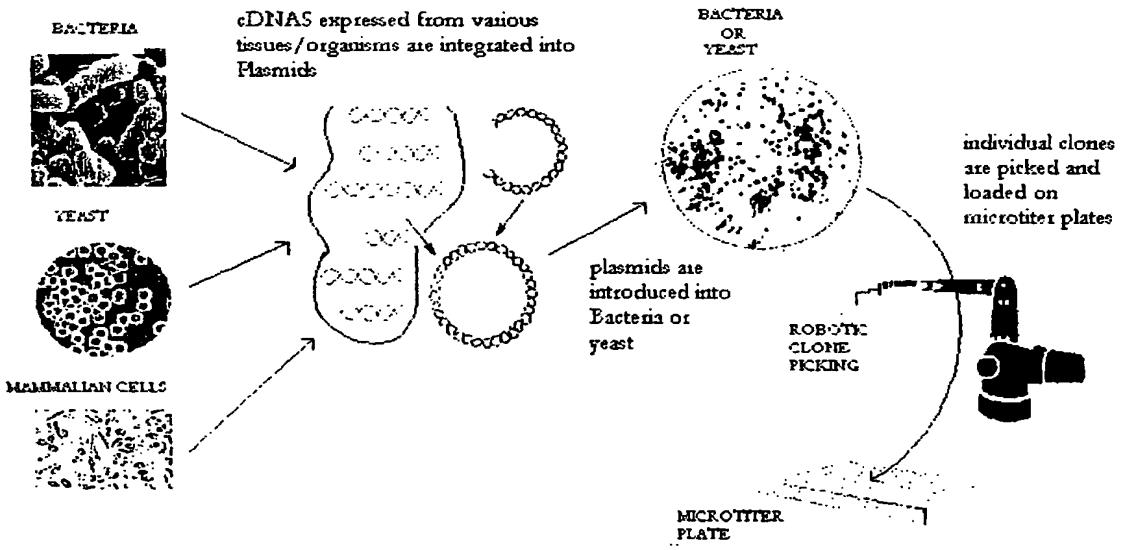

(57) Abstract: The present invention is related to a method for high-throughput lead and the use of microcalorimetric devices therein.

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METHOD AND TECHNOLOGY FOR HIGH-THROUGHPUT LEAD PROFILING

Field of the invention

[0001] The present invention is related to a method for high-throughput lead profiling and the use of microcalorimetric devices therein.

State of the art

[0002] In response to increased economic pressures, research-based pharmaceutical companies need to accelerate their drug discovery and development processes. The most critical factor in the development of new significant NCEs is the preclinical testing, in which an average of sixty percent of NCEs produced by drug discovery fail. Of the forty percent that eventually undergo human clinical testing, less than one tenth ultimately come through clinical trials and become marketed products. Thus, the amount of lead compounds that are optimized to development candidates and that eventually reach early clinical trials is still alarmingly high.

[0003] Most of the failures are due to undesired or unpredicted interactions with non-specific targets. This can result in bad or no transport across membranes, rejection of drugs by Multiple Drug Resistance Proteins or P-glycoproteins, transformation or destruction of the drug by metabolic enzymes, toxicity as drugs affect essential targets for cell survival, side effects of the drugs caused by interaction with unpredicted receptors or enzymes.

[0004] The high level of attrition in compounds developed as leads has prompted many companies into applying technologies such as high throughput target profiling in early drug discovery, integrated in a strategy to assess the "drug-like characteristics" of chemical leads, with the object of predicting and avoiding those failures that occur in clinical trials. At the same time these strategies and technologies can also be used in the optimization of chemical leads into NCEs.

[0005] This pharmaceutical profiling, integrating ADME and pharmacokinetic information into early and mid-discovery, optimally includes analysis of drug solubility and permeability (commensurate with route(s) of drug administration.), stability, metabolism liabilities, pharmacokinetic parameters including blood concentrations, distribution and half-life, appropriate formulation excipients and toxicity and undesirable side effects.

[0006] Toxicity studies are performed mainly by assaying the chemical entity (hits, leads or drugs) on a panel of different receptors and enzymes, optionally also on whole-cell systems. A limited number of companies offer lead profiling, some for up to 300 targets. However, the maintenance and updating of hundreds of different assays on many targets is very costly. At the same time the generation of a representative number of targets that can be screened is a problem. The ability to do profiling of new chemical entities against all (or at least a large number of) potential targets however, would increase the chance of identifying unpredicted interactions. Additionally, the availability of a screening method which allows the (rapid) identification of an interaction between a chemical entity and large number of targets could

dramatically reduce drug development failures by enabling pharmaceutical lead profiling at an early stage.

[0007] The most sensitive assays for determining interaction (i.e. binding and/or activity) of a chemical with a target rely on radioactivity- or fluorescence-based detection methods. These methods have obvious disadvantages when working with small molecules and/or living cells. Alternatively, most physiometers do not allow sensitive testing on a high-throughput scale.

[0008] The present invention discloses a method to perform high throughput screening of chemical entities for their activity on multiple targets, based on micro-calorimetry.

[0009] Calorimetry in general is a measurement principle that detects all processes that occur in a reaction vessel. Calorimetry has several advantages: calorimetry is the most general detection principal, since most processes, physical, chemical or biological are accompanied by changes in heat content. This can be useful in the analysis of very complex processes, because it is more likely that unknown phenomena will be discovered. Moreover, the change of temperature of a reaction volume is dependent upon the concentration of the reagents, not upon the absolute quantity, offering the possibility of miniaturisation. Additionally, calorimetric methods are not dependent upon the sample form (the sample can be solid, liquid, gaseous, or any combination thereof, and neither colour, optical transparency, nor absence of suspended matter are requirements). Calorimetric measurements are non-invasive (it is not necessary to disturb the biological system, for example, by radiation). It even allows on-line monitoring of living cells over a longer period of time to obtain kinetic data. It is non-destructive towards the sample (no need for fluorescent markers or fixation procedures).

[0010] The state of the art are devices capable of measuring small temperature differences between relatively large reaction volumes (typically 0.12 ml to 100 ml). SETARAM (Caluire, France) is a provider of calorimetric devices. These products are relatively large batch systems, mainly intended for applications in the biochemical field. The ITC® (Isothermal Titration Calorimeter, MicroCal, LLC, Northampton, USA) has a cell volume of 1.3 ml (and a detection limit of 400 nW). The TAM® (Thermal Activity Monitor, Thermometric, Järfälla, Sweden) has a cell volume of 4 ml (and a detection limit of approximately 100 nW). These micro-calorimeter batch systems are non-compatible with high-throughput requirements due to the relatively large volumes, the apparatus' closed structure and long cycle times (typical cycle times are 2 to 3 hours).

[0011] Other devices known in the art are capable of detecting small absolute temperature changes in extremely small reaction volumes. Commercially available micro-calorimetric sensors have also been described, using thermopiles and thermistors (Xensor Integration, Delft, The Netherlands). A main disadvantage of these devices is that they are configured in such a way that the reference temperature is the temperature of the silicon border. This leads to problems concerning baseline stability and common rejection mode since no differential measurements are used. Additionally, these devices are not compatible with standard robotics used in high throughput screening since no recipients are integrated.

Aims of the invention

[0012] The present invention aims to provide a new method for high-throughput screening of chemical entities on a large number of targets.

Summary of the invention

[0013] The present invention comprises a method for high-throughput screening of chemical entities on a large number of targets.

[0014] According to a specific embodiment of the present invention the large number of targets encompasses a collection of cells or cellular proteins.

[0015] According to one aspect of the invention said method comprises generating a cDNA expression library of an organism in a host and testing the chemical entity against individual clones of this expression library. According to a preferred embodiment of the invention the cDNA expression library is a human cDNA expression library.

[0016] According to another aspect of the invention the method comprises screening for the interaction of the chemical entity and the target by way of calorimetry. In a preferred embodiment said calorimetric measurement is performed using a microcalorimetric device comprising a differential heat detection means. Most preferably this differential heat detection means is a thermopile.

[0017] Thus, according to another aspect of the invention, the use of a microcalorimetric device is disclosed for screening the interaction of a chemical entity with a large number of targets, in order to determine the pharmacological profile of this chemical entity.

[0018] In a preferred embodiment the device comprises at least two array elements on a supporting substrate. The elements, separated from each other by a first isolation zone (arranged to thermally isolate said array elements), comprise:

- A first and a second receiving zone, both arranged to provide a contact between samples and stimuli,

- A heat detection means, and
- A second isolation zone between the first and second receiving zone.

Preferably the first and second isolation zones are formed by at least part of the supporting substrate. Most preferably the supporting substrate of the device has sufficient strength to support the array device and the first and second isolation zones are arranged to thermally isolate the array elements and the first and second receiving zones.

[0019] The device of the present invention for use in pharmacological profiling of chemical entities is preferably dimensioned as a standard 96, 384, 1536 or 6144-well microtiterplate.

[0020] Thus the present invention encompasses the use of the device described above in the screening of the interaction of a chemical entity on multiple targets. More specifically, the described device is used in pharmacological profiling of hits or leads in drug development.

[0021] Another aspect of the present invention is a calorimetric measuring method for measuring the interaction of a chemical entity with multiple targets for use in drug-profiling, said method comprising the steps of:

- Providing different targets in the reaction vessels of a calorimetric device,
- Adding the chemical entity to the targets in the test reaction vessels, and,
- Measuring the heat released by the interaction between the chemical entity and the targets.

[0022] Preferably the calorimetric device is a device capable of measuring very small temperature increases in multiple reaction vessels simultaneously. Most preferably,

the calorimetric device is an array device as described herein.

[0023] It will be understood that particular embodiments of the invention are described by the dependent claims cited herein.

Short description of the drawings

[0024] Figure 1 represents how a cDNA Library can be obtained.

[0025] Figure 2 represents a microcalorimetric device according to the present invention.

[0026] Figure 3 represents a microcalorimetric device wherein the membrane is part of the substrate.

[0027] Figure 4 represents an illustration of a screening operation using a cDNA library.

Detailed description of the invention

[0028] The present invention discloses a method for screening the interaction of a chemical entity on a large number of targets.

[0029] A chemical entity as used herein relates to a chemical molecule or compound (hits, leads or drugs) obtained in drug development. Preferably the chemical entity corresponds to a hit or lead which has not yet been tested in pre-clinical or clinical trials.

[0030] A target as used herein relates to a DNA, protein, multi-protein complex, cellular structure, cell organelle, cell or tissue or culture thereof with which a drug can have a direct interaction. Thus the term "target" as used herein in fact refers to "non-specific" or non-intended targets, i.e. other molecules than the selected target the interaction with which was used as a criteria for selection. Alternatively, such targets will also be referred to as "samples". Preferably, according to the

present invention, the interaction of a chemical entity is screened on a maximal number of samples, corresponding to most or all possible non-specific targets with which the chemical entity, when administered to a human or animal as a drug can interact. Thus, the set of samples can comprise a variety of cells (i.e. in culture) or cellular structures from different organs. Such cells include cancer cells or cells that are genetically modified. Alternatively, a collection of proteins (such as, i.e., blood proteins) or receptors. According to a preferred embodiment of the present invention, a maximal amount of possible samples is screened by using a cDNA expression library to generate a collection of samples.

[0031] cDNA Libraries can be obtained according to standard recombinant DNA technology and essentially comprises the following steps: mRNA is prepared (characterized by a long poly-A tail at the 3' end, so it can be extracted using an oligo-dT column) after which oligo-dT primers are then annealed to the poly-A tail, which facilitates synthesis of a DNA copy through the use of reverse transcriptase. The original RNA strand is then removed by alkaline hydrolysis. DNA polymerase is used to synthesize the second DNA strand and this is initiated at various sites through the use of random hexamer nucleotide primers. Use of a DNA ligase covalently seals the remaining breaks. The cDNA may be inserted into a phage vector, such as lambdagt10, in order to create a library. These phage particles can be propagated through plaque formation on a bacterial lawn, alternatively cDNAs can be inserted in plasmids which are then introduced into bacteria or yeast by transformation. Genomes from various organisms from bacteria to man, including yeast and protozoa can be obtained from small isolates and subsequently expressed for screening. Genomes or cDNA libraries can be purchased

separately from public banks (ATCC, etc...) or commercial suppliers. Each clone can be isolated and grown in a microplate format. Such a library can contain from thousands to hundreds of thousand isolates. Mother plates are replicated with a 96 or 384 pin tools. The replicated isolates can then be grown. The cells or bacteria can be disrupted with a specific lysis buffer or by ultrasound. Such protein lysates are then ready to be screened (Figure 1).

[0032] The present invention discloses a method for screening the interaction between a chemical entity and a large number of samples. For the purpose of this invention, the interaction between the chemical entity and the sample is referred to as an "event". Such an event can induce different signals such as change of heat or enthalpy, change of ionic concentrations of different ions, etc.... Thus, the occurrence of an event can be measured by pH-metry or calorimetric methods measuring heat and/or irradiation.

[0033] According to a preferred embodiment of the invention, the interaction is measured by calorimetry, more specifically by an apparatus capable of measuring small temperature changes generated in multiple reaction vessels, i.e. wherein multiple heat detection means are set up in an array. According to a preferred embodiment of the invention, the device for use in screening of the interaction between a chemical entity and multiple samples, is a device as described hereafter.

[0034] The apparatus comprises an array device, said array device comprising on a supporting substrate at least two array elements that are separated from each other by an isolation zone, said array elements comprising:

- A receiving zone arranged to provide a contact between a sample and a chemical entity,

- A heat detection means arranged to perform a measurement of heat between said receiving zone and a reference, and
- Said isolation zone being formed by at least part of said supporting substrate,

Characterised in that said supporting substrate has sufficient strength to support said array device and said isolation zone is arranged to thermally isolate said array elements

[0035] The array device comprises a substrate with at least two substantially identical reaction vessels or receiving zones which preferably form a part of the substrate. The reaction vessel is used for retaining the chemical entity and for providing contact between the chemical entity and the protein or cell sample.

[0036] The device further comprises at least one heat detection means. Said heat detection means can be a heat detection means selected from the group consisting of a thermistor, diode, IR detection means, CCD camera, a thermopile. Preferably said heat detection means is a differential heat detection means integrated in the substrate for monitoring changes in heat content or enthalpy generated by the chemical entity upon being contacted with the sample. The differential heat detection means is operatively associated with the first and the second reaction vessel (or receiving zones) so that a differential measurement between the first reaction vessel (the "reference" reaction vessel) and the second reaction vessel (the "measurement" reaction vessel) can be performed. The first and the second reaction vessel are preferably neighbouring reaction vessels.

[0037] The differential heat detection means (DHDM) of the device is arranged to perform a differential measurement of heat between two reaction vessels (or

receiving zones). The first receiving zone is used as a reference while in the other an event is generated. When a change of heat or enthalpy is generated, the thermal input signal is converted into a differential electrical output signal. Preferably, the reference sample is substantially equivalent to the test sample, so that heat capacities and surface relationships (which cause condensation or evaporation) do not influence the measurement.

[0038] The array device preferably has the format of a standard microtitre plate, such as, but not limited to a 96-well, 384-well, or 1536-well microtitre plate (see table 1). Alternatively, the design of the array can be customised for integration in any high-throughput screening system. This allows the use of the array in standard robotics used in drug screening. In a preferred embodiment of the invention, the array is a sensor-arrayed chip with the footprint of a standard 96-well titre plate, and thus compatible with pharmaceutical robotics for dispensing and titre plate handling. The distance between adjacent wells in this format is 9 mm. For formats derived from this reference, the inter-well distance is 9 mm divided by the miniaturisation factor. The miniaturisation factor is defined as

$$m = \sqrt{\frac{n\_wells}{96}}$$

with n_wells the number of wells.

[0039] The differential heat detection means in the device used according to a preferred embodiment of the invention is a thermopile, consisting of a set of 2 temperature sensitive means with a differential read out. Said thermopile has a hot and a cold junction, which are operatively associated with the first and the second

reaction vessel respectively. The hot and cold junction are thermally isolated one from another.

[0040] A thermopile is made up of a number of thermocouples, electrically connected in series, thermally connected in parallel.

[0041] The main advantages of a thermopile are:

- The thermopile is a self-generating offset-less device, as the heat flowing through it supplies the power for the output signal. As a result there is no offset drift and no interference caused by power supplies.
- The sensitivity of the thermopile is hardly influenced by variations in the electrical parameters across the wafer or by the temperature.

[0042] The thermopile can be optimised in terms of dimension and number of thermo-electric strips.

[0043] The substrate of the device used according to a preferred embodiment of the invention can be, but is not limited to, one of the group consisting of silicon, silicon oxide, silicon nitride, silicon oxynitride, polysilicon, porous silicon, plastic, polymer (also rubber, PVC, etc...), biodegradable polymer, glass, quartz, ceramics, aluminium oxide, agar, biological material, and rubber. When the substrate is a material compatible with semiconducting processing, the integration of sensors and fluidics on the same chip is facilitated. This opens the way to small sample volumes and inert reaction vessels, since no reaction with materials or adsorption of materials occurs. Said substrate should be made such that it has sufficient strength to support said array

[0044] The reaction vessel (or receiving zone) of the device used in the context of the present invention refers to a means or carrier of sample and/or chemical entity. More specifically, if the sample used is biological

material, such as a cell culture, the reaction vessel will preferably be of a format capable of containing fluids. Preferably, the reaction vessel is capable of handling volumes in the range from 0 ml to 100 ml, more preferably from 0 ml to 7 ml, especially preferably from 0 ml to 5 ml, most preferably from 0.1 nl to 1 ml. Preferably, said reaction vessel has a maximum volume of 5 ml. Furthermore, the reaction vessel is operatively associated with the differential heat detection means. This means that there is a thermal coupling between the reaction vessel and the temperature sensitive part of the differential heat detection means.

[0045] In a preferred embodiment of the device used according to the present invention, the reaction vessel (or receiving zone) is formed by a recess in a substrate. Said reaction vessel can also be an area on the substrate e.g., the whole substrate or parts of the area of the substrate can be chemically or physically modified in such a way that they can selectively hold one or more of said sample, medium, and chemical stimulus.

[0046] Alternatively, the reaction vessel (or receiving zone) can be a microvessel made of a material, such as but not limited to, metal, steel, silicon, silicon oxide, silicon nitride, silicon oxynitride, polysilicon, porous silicon, plastic, polymer (also rubber, PVC, etc...), biodegradable polymer, glass, quartz, ceramics, aluminium oxide, agar, biological material, and rubber, which is either placed on top of, or is hanging above the substrate. For instance, the reaction vessel can be formed by a needle form dispenser. Preferably, the distance between the substrate and the reaction vessel is not larger than the distance between two adjacent reaction vessels.

[0047] As described above, each reaction vessel (or receiving zone) is surrounded by an "isolation zone", which functions as a thermal isolation zone.

[0048] The membrane of the device used according to a preferred embodiment of the present invention is a part of the device which provides thermal isolation. The membrane can be part of the substrate or can be a second substrate.

[0049] Alternatively, when the reaction vessel is formed by complete etches through the substrate extending from the first surface to the second surface, a thin membrane can be formed for covering said recess at a first side or at the second side of the substrate.

[0050] The membrane can be made of the substrate material or of another material. The membrane can be formed, by methods such as, but not limited to, growing a membrane layer on the substrate or bonding a membrane.

[0051] The membrane can also be made of another material than the substrate material. For instance, a thin layer of the membrane material can be formed on the substrate material. The recess can be formed in the substrate by conventional micromachining techniques. The recess can extend from the first surface of the substrate until the membrane. When e.g. dry etching techniques are used, the membrane material and etch chemistry can be chosen such that a recess is etched in the substrate and that the etching process selectively stops on the membrane.

[0052] The thickness of the membrane can be between 1 μm and 1 cm, preferably between 1μm and 1 mm, most preferably between 10 μm and 0.1 mm. The thickness depends on the membrane material, and consequently on the thermal isolation of the membrane material. For example, the

thickness of a silicon oxide membrane can be lower than 10 µm. For glass, the thickness can be between 1 and 5 mm.

[0053] Any of the above mentioned materials can be perforated and/or patterned material such that a perforated membrane or sieve is created. When a liquid is supplied to the reaction vessel, oxygen molecules can escape through these openings. Additionally or alternatively, the sieve serves to encapsulate biological material. Sieves are useful when specific elements, like cells or tissue, must be entrapped inside the physiometer, while maintaining (periodic) contact with a nutritive medium.

[0054] The size of the openings of the sieve is determined by the function of the sieve. For instance, when desiring cellular entrapment, the openings will preferably have a diameter of about 6 µm, while for tissue entrapment the openings will preferably have a diameter of about 20µm. It is understood that the size of the openings can be optimised depending on the sample used.

[0055] The sieve is preferably made of low stress material (1.2 µm plasma oxide). In places where sensors are positioned, other structural layers are present.

[0056] When the reaction vessel is a recess in the substrate, the openings will extend from the bottom of the recess to the second surface of the substrate. When the reaction vessel is part of the first surface, the openings will extend from this first surface to the second surface. Said membrane can also be a suspended membrane.

[0057] The device or array of devices can further comprise a membrane which covers it. This membrane can also be called "lid". The lid can be made of at least one material such as, but not limited to, metal, steel, silicon, silicon oxide, silicon nitride, silicon oxynitride, polysilicon, porous silicon, plastic, polymer

(also rubber, PVC, etc...), biodegradable polymer, glass, quartz, ceramics, aluminium oxide, agar, biological material, and rubber.

[0058] The same sieve structure as described for the membrane can be found in the lid. The lid can cover the reaction vessel. In this way, cell or tissue can be entrapped.

[0059] The device used according to a preferred embodiment of the invention, can further comprise an extra detection means for screening the interaction between a chemical entity and the samples. This extra detection means can be a potentiometric sensor (such as, but not limited to, a Light Adressable Potentiometric sensor or "LAPS"), a FET device, a diode, interdigitated electrodes ("IDES"), a reference electrode, a working electrode and/or an impedance spectroscopic device. This extra detection means can be placed such that it is operatively associated with said reaction vessel. For example, said extra detection means can be integrated in the substrate. When the reaction vessel is a recess in the substrate, the extra detection means can be integrated in e.g., the side walls or the bottom wall of the reaction vessel. When the reaction vessel is an area on the substrate, the extra detection means is preferably integrated in the substrate. The number of extra detection means per reaction vessel is not limited.

[0060] Furthermore, in the context of the present invention it is envisaged that, in particular circumstances in determining the interaction between a chemical entity and a sample, it may be required to set the device at a given temperature. Therefore, the device used in the context of the present invention may further comprise a calibration means for thermosetting said device. The calibration means can be a tuneable electrical power

generating means such as e.g. a resistor or a thermopile or a temperature sensitive means such as a resistor, diode, thermopile or a microprocessor that drives the power generator in such a way that a pre-determined temperature is obtained.

[0061] Additionally, the device used in the context of the invention can comprise a supply means to fill the reaction vessels in an active (e.g. pressure or suction force) or passive (e.g. capillary force) manner. The supply means can be fabricated in a material such as, but not limited to silicon, silicon oxide, silicon nitride, silicon oxynitride, polysilicon, porous silicon, plastic, polymer (also rubber, PVC, etc...), biodegradable polymer, glass, quartz, ceramics, aluminium oxide, agar, biological material, and rubber. In a preferred embodiment of the invention, the supply means has the same dimensions and layout as the device, except the heat detection means is omitted. The supply means can optionally have external or internal (e.g. made by micromachining) pumps and valves. Alternatively, said supply means can also be an industrial dispenser with or without external pumps and valves. The supply means can also be a custom-made dispenser with or without external or internal (e.g. made by micromachining) pumps and valves.

[0062] Optionally, the apparatus used in the context of the present invention comprising an array of devices makes use of capillary forces to supply the solutions to the reaction vessel(s).

[0063] The device of the present invention can further comprise read-out electronics such as (pre-) amplifying, multiplexing, filtering and/or buffering circuitry.

[0064] For calibrating said the calorimetric measurement, resistors, other than those used for

thermosetting, are used as calibration means and are situated in the walls of the reaction vessels. The calibration resistor is designed to be able to produce dissipation heat in the same range as the produced biological power.

[0065] Techniques used to fabricate such an array device can be, but are not limited to, molding or micro-electronic processing. Micro-electronic processing offers specific advantages such as cost reduction if mass production is envisioned. Further, extreme miniaturisation is possible, the degree of which is limited by e.g. lithography. A high degree of parallelism is possible, making high-throughput feasible. Moreover, the detection limit of the system can be decreased considerably because fluidics are integrated on the same chip as the sensing element, reducing the length of the thermal path from reaction to sensing site. In addition, the detection limit of the system can be decreased even more if pre-amplifiers are integrated on the chip.

Example: Identification of proteins interacting with a compound issued from a pharmacological screening

[0066] Compound C is selected as a lead molecule, in a drug development proces involving the screening of a commercial compound library for specific interaction with a target, receptor R.

[0067] In order to obtain information on the possible interaction of this compound with non-intended targets, it is screened against proteins obtained from an animal or human expression library. Interaction between the compound and proteins is measured via microcalorimetry. Once positives have been identified, the corresponding cDNA is sequenced.

a) development of a sample library

[0068] A cDNA library is prepared from DNA isolated from human cells according to classical recombinant DNA technology and introduced in a phage vector in a bacterial host. Each clone is isolated and grown in a microplate format. Mother plates are replicated with 96- or 384- pin tools. The replicated isolates can then be grown. The bacteria are disrupted with a specific lysis buffer or by ultrasound. The protein lysates so obtained can be used for screening (Figure 1).

b) Screening

[0069] Screening is performed by way of a microcalorimeter based on a device which allows the detection of extremely small temperature differences between two reaction vessels, which are biocompatible and of which an array can be formatted which has the footprint of a microtitre plate (Figure 2).

[0070] The use of silicon as substrate material provides the possibility of integrating the sensors and fluidics on the same chip, so that sample volumes can be minimalized (minimal dead volume). Additionally, the reaction vessels which are recesses in the substrate are inert, as no reaction or sorption of materials occurs. As differential heat detection means a thermopile is integrated in the substrate. Additionally, 2 resistors are integrated in the walls of the reaction vessels as thermosetting means. The membrane is perforated to obtain a sieve with openings 6 μm wide, to enable cellular entrapment.

[0071] The measurement is performed essentially as follows (Figure 4):

- All reference reaction vessels (blanks) of the microtitre plate contain only screening buffer
- All measurement reaction vessels contain the compound C (prepared in a screening buffer)
- Temperature is monitored in real time.
- A number of measurement reaction vessels are each loaded with a sample of protein lysate obtained from a clone of the cDNA expression library, containing (as of yet) unidentified proteins. Control reaction vessels are loaded with the receptor R
- Temperature is monitored in real time.

[0072] Alternatively, the protein and reference samples are loaded in the reaction vessels first, and the chemical entity is added after a first real time temperature measurement.

[0073] Presence of a protein in sample of the protein lysate which interacts with compound C causes an interaction. The reaction enthalpy heats up the reaction vessel and this is detected by the DHDM. Additionaly, any accompanying change in proton concentration is detected by the pH detector. The coordinates of the well in which a thermal signal is detected allow the identification of the corresponding bacterial clone.

c) Hit processing

[0074] Positive reaction vessel coordinates are registered into a database; the corresponding cDNA is then automatically sequenced. The DNA sequenced is then analyzed using bioinformatics softwares to identify the corresponding amino acid sequence, which is finally compared to existing information on public databases. This information can be

- the identity of the protein

- the function of the protein
- the hypothetical function of the protein based on homology with a known amino acid sequence.

5

CLAIMS

1. A method for high-throughput screening of chemical entities on a large number of samples, said method comprising:

- generating a cDNA expression library of an organism in a host; and
- Testing the chemical entity for the interaction with the protein expression products of individual clones of the is expression library.

2. The method of claim 1, wherein said cDNA expression library is a human cDNA expression library.

3. The method of claim 1, wherein said interaction between said chemical entity and said protein is measured by way of calorimetry.

4. The method of claim 3, wherein said calorimetric measurement is performed by an apparatus comprising an array device, whereby each device comprises a heat detection means.

5. The method of claim 4, wherein said microcalorimetric device is an array device comprising a supporting substrate at least two array elements that are separated from each other by an isolation zone, said array elements comprising:

- a reference and a measurement reaction vessel with a cross-section of less than 10 mm,
- a heat detection means arranged to perform a measurement of heat between said measurement reaction vessel and said reference reaction vessel;

wherein said isolation zone is formed by at least part of said supporting substrate; and

wherein said supporting substrate has sufficient strength to support said array device.

6. The method of claim 5, wherein said heat detection means is a differential heat detection means.

7. The method of claim 6, wherein said differential heat detection means is a thermopile.

8. The method of claim 5, wherein said reaction vessels further comprise a resistor.

9. The use of a microcalorimetric device for screening the interaction of a chemical entity with a large number of samples, in order to determine the pharmacological profile of this chemical entity.

10. The use of claim 9, wherein said microcalorimetric device is an array device comprising a supporting substrate at least two array elements that are separated from each other by an isolation zone, said array elements comprising:

- a reference and a measurement reaction vessel having a cross-section of less than 10 mm,
- a differential heat detection means arranged to perform a measurement of heat between said measurement reaction vessel and said reference reaction vessel;

wherein said isolation zone is formed by at least part of said supporting substrate; and

wherein said supporting substrate has sufficient strength to support said array device.

11. The use of claim 10, wherein said large number of samples corresponds to the products of a cDNA expression library.

12. The use of claim 11, wherein said cDNA expression library is a human cDNA expression library.

13. A calorimetric measuring method for measuring the interaction of a chemical entity with multiple samples for use in drug-profiling, said method comprising the steps of:

a) Providing different samples in the measurement reaction vessels of a calorimetric device,

b) Adding the chemical entity to the samples in reaction vessels, and,

c) Measuring the heat released by the interaction between the chemical entity and the samples.

14. The method of claim 13, wherein said samples are generated by a cDNA expression library.

15. The method of claim 14, wherein said expression library is a human cDNA expression library.

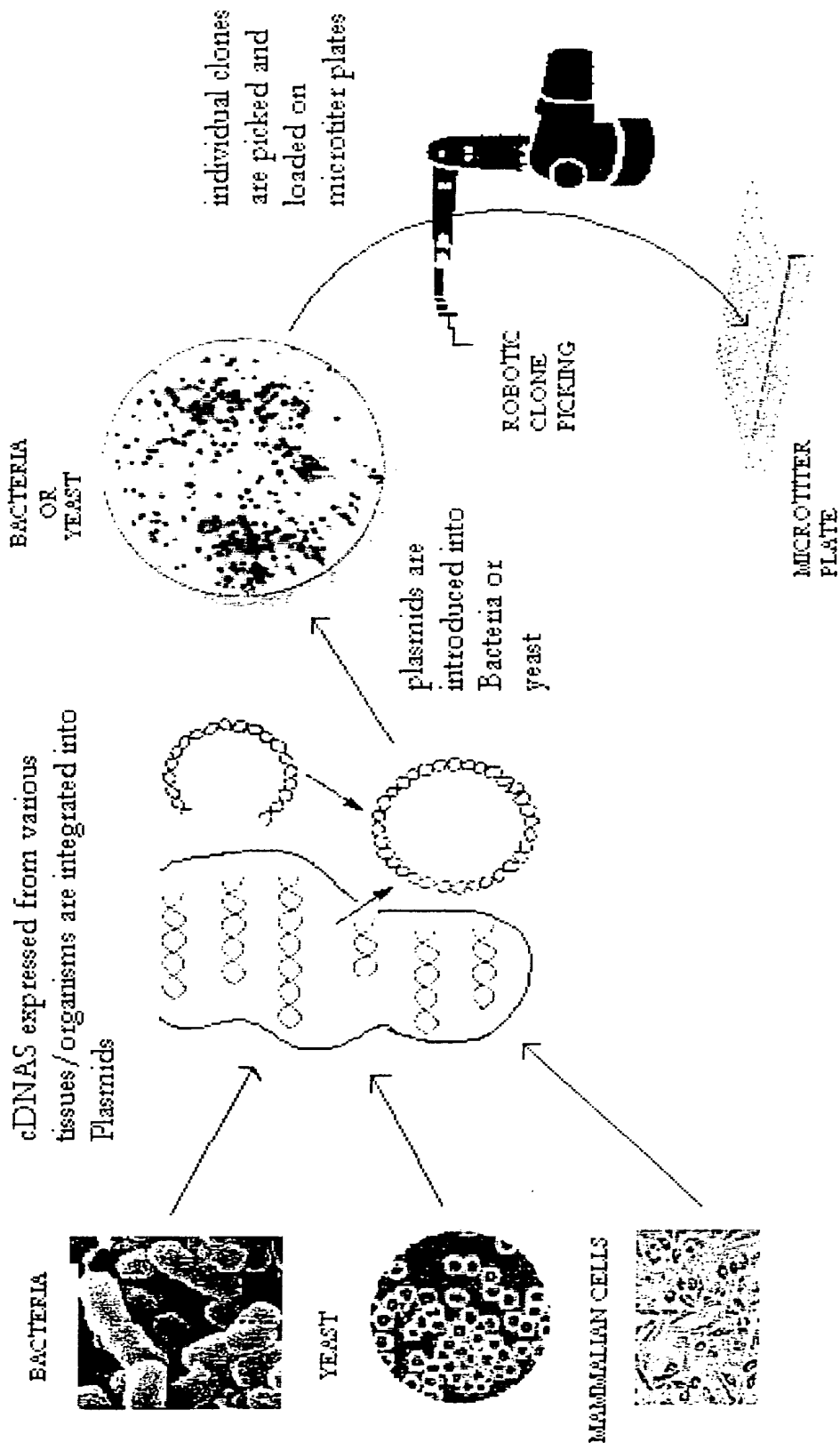

FIG. 1

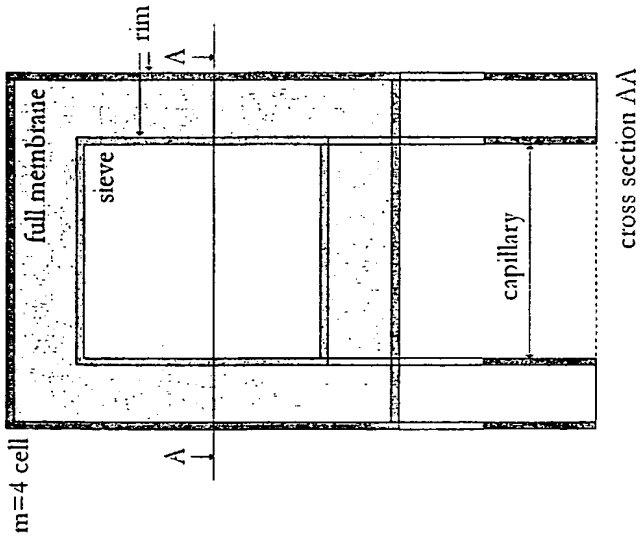
m=4 cell
full membrane
sieve
rim
A
A
capillary
cross section AA

FIG. 2

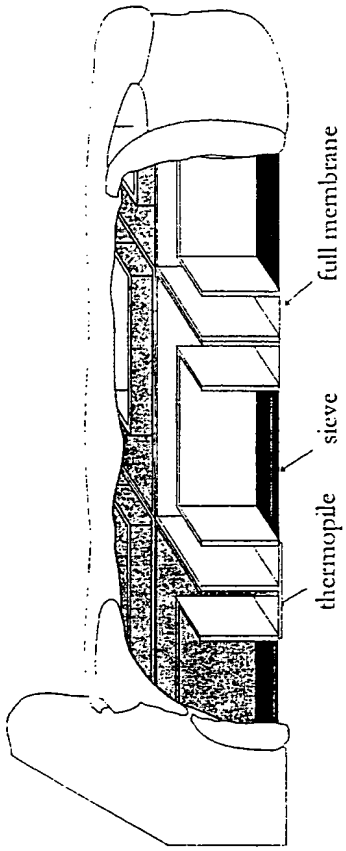
full membrane
sieve
thermopile

FIG. 3

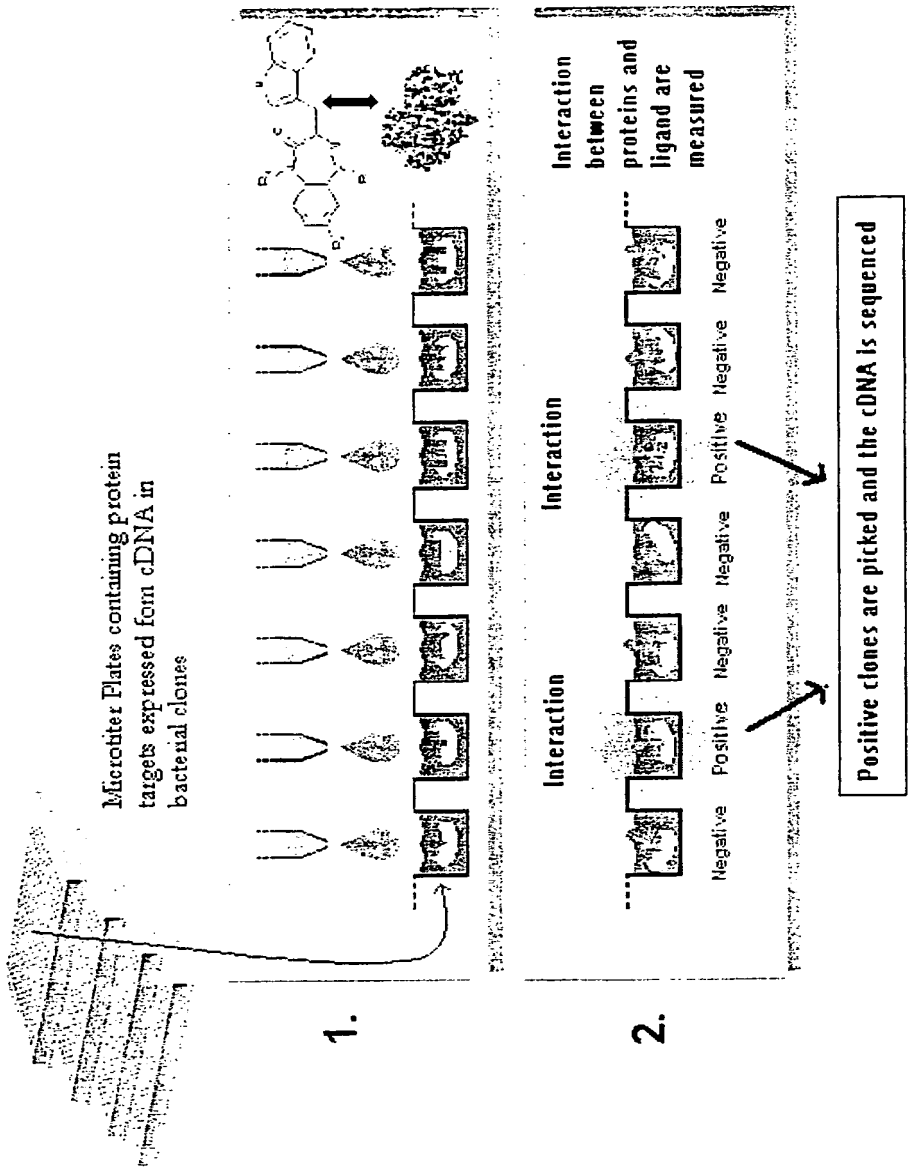

FIG. 4

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Published:
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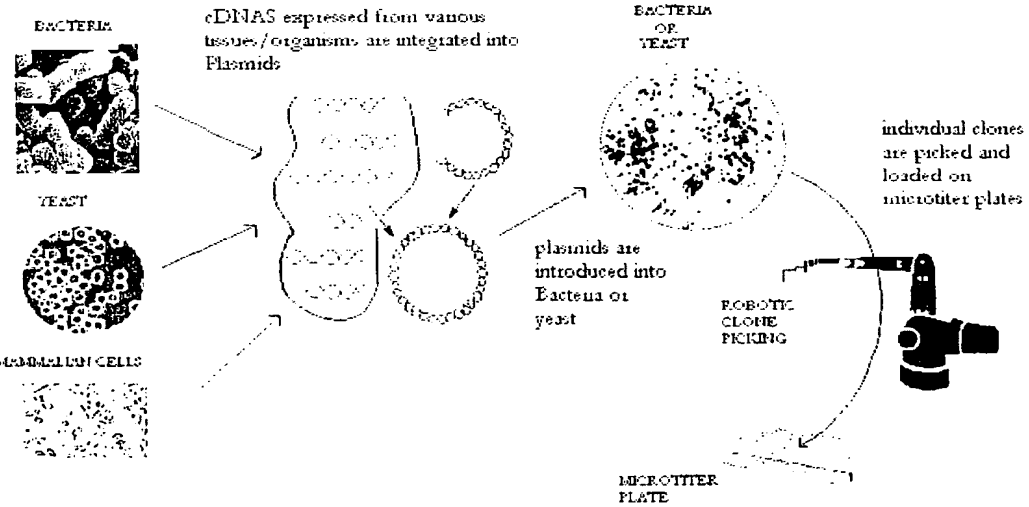
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR HIGH-THROUGHPUT LEAD PROFILING



(57) Abstract: The present invention is related to a method for high-throughput lead and the use of microcalorimetric devices therein.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/BE 01/00080

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, INSPEC, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	the whole document	3-8,14, 15
X	US 5 255 976 A (CONNELLY PATRICK R) 26 October 1993 (1993-10-26)	9,13
Y	column 1, line 5 -column 2, line 39; figure 1	3,10,14, 15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 March 2002	02/04/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Savage, J

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

Int ional Application No
PCT/BE 01/00080

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